COMBINATION EFFECTS OF SHOSAIKOTO (CHINESE TRADITIONAL MEDICINE) AND PREDNISOLONE ON THE ANTI-INFLAMMATORY ACTION

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Effects of combined use of Shosaikoto (one of the famous crude Chinese medicines) and prednisolone were examined in anti-inflammatory effects, using the carrageenan edema and the cotton pellet methods. Shosaikoto and prednisolone were orally given to rats or mice at the dose of 1.1 g/kg (corresponding to 10 times the usual human dose) for the former and 1.0, 4.0 and 16.0 mg/kg for the latter. Shosaikoto showed the mild anti-inflammatory action and significantly increased the anti-inflammatory effect of prednisolone in both experimental models. In cotton pellet method, combined use of Shosaikoto inhibited the decrease of adrenal weight induced by prednisolone.

To clarify the mechanism of combined effects of prednisolone and Shosaikoto, the effects of Shosaikoto on the blood prednisolone level and on the secretion of endogenous glucocorticoid (corticosterone) were investigated by high performance liquid chromatography. The blood prednisolone level 20 min after the combined administration of Shosaikoto with prednisolone, 16 mg/kg, was about 2 times comparing with that of single administration of prednisolone. The half life period of blood prednisolone after the single administration of prednisolone was about 2 times comparing with that of the combined administration of Shosaikoto with prednisolone. On the other hand, the single administration of Shosaikoto increased the blood corticosterone level 1 h after the administration with significant manner. The increasing activity of Shosaikoto on the anti-inflammatory effect of prednisolone may be explained by its plural actions.

Keywords — Kampohozai; Chinese traditional medicine; Shosaikoto; prednisolone; anti-inflammatory; corticosterone; HPLC

INTRODUCTION

Chinese traditional medicine is a drug therapy which has been systematized from the clinical experiences accumulated over some thousand years in China. But at present, it is not evaluated as high as western medicine. The main reason for this would lie in the use of a mixture of natural crude drugs which ensures the quality not so much as a western drug and their ingredients would influence mutually. The physically, chemically and pharmacologically complicated nature of this medicine makes the scientific analysis difficult.

Shosaikoto is one of the chinese traditional medicines (Kampohozai), which is a mixture of seven crude drugs, Bupleuri Radix, Glycyrrhizae Radix, Zizyphi Fructus, Zingiberis Rhizoma, Ginseng Radix, Pinelliae Tuber and Scutellariae Radix. Bupleuri Radix which is a main component of Shosaikoto contains pharmacologically active glycosides, saikosaponin a, c and d as main saponins. The structures of saikosaponins and their aglycones, saikogenins, have been reported by Shibata et al.\(^{1,2}\) and Kubota et al.\(^{3-5}\) Their anti-inflammatory and other pharmacological actions were studied by Oura et al.\(^{6,7}\).
et al.,8) Takagi et al.9) and Arichi et al.10,11) Based on the above reasons, Shosaikoto is expected to show anti-inflammatory effects due to the actions of saikosaponins and saikogenins.

On the other hand, drug therapy of chronic inflammatory diseases is widely performed with steroids, non-steroid drugs, immunosuppressors and immunoregulators these days. But these drugs have to be applied with care for adverse effects and have not yet been established as a decisive treatment in spite of intensive studies in a wide range. Recently, Arichi et al.12) have reported that the combination of some Saikozai which possesses Bupleuri Radix as a component and steroid showed good results in making easy the seceding from the steroidal treatments in patients who are treated with steroids for a long period. These facts indicate that the combination of some Kamphozaizai and steroid shows some significant effects on inflammation or immune response.

In the present study, we examined the effects of Shosaikoto on a steroid preparation in aspects of increasing the anti-inflammatory effects of the prednisolone preparation. Further more, the effects of Shosaikoto on the absorption of prednisolone into blood and on the secretion of corticosterone from adrenal gland were investigated to elucidate the mechanism of action of Shosaikoto on the increase of the anti-inflammatory effects of prednisolone.

EXPERIMENTAL

Animals — Male Wistar rats weighing 180—200 g and male ddY mice weighing 20—25 g were used. They were kept in air conditioned room (24 °C) under a light-dark cycle (light phase, 06:00—18:00 o’clock) and given commercial diet and water ad libitum, unless otherwise specified.

Agents — Prednisolone (Nakarai Chem. Co.) was suspended in water and administrated orally.

Preparation of Shosaikoto — Glycyrrhizae Radix (3.0), Zizyphi Fructus (3.0), Zingiberis Rhizoma (1.0), Ginseng Radix (3.0), Pinelliae Tuber (8.0), Scutellariae Radix (3.0) and Bupleuri Radix (8.0) were added in water (480 ml), boiled for 1 h and concentrated to 120 ml (the numbers indicated the ratio of composed crude drugs by the dry weight (g)). The concentrated decoction was lyophilized to give 6.6 g of extracts.

Powdered extracts, 1.1 g/kg (ten times the human dose), were administered orally by dissolving in 0.2 ml of water for mice and 2.0 ml of water for rats. To evaluate the quality of Shosaikoto used, the main components were determined by high performance liquid chromatography (HPLO).13-16) The results were: saikosaponin a 2.28 ± 0.03, saikosaponin b2 2.09 ± 0.14, glycyrrhizin 52.7 ± 0.40 (means ± S.E., mg/human dose/d, N = 3).

Anti-inflammatory Activity on Carrageenan-Induced Hind Foot Edema in Rats — Male Wistar rats weighing 180—200 g were used as experimental animals to induce foot edema with λ-carrageenan (Shigma Chem. Co.).16,17) Groups of 6—9 rats were used. λ-Carrageenan (1.0% in physiological solution, 0.05 ml) was injected s.c. under the planter surface of right hind paw 1 h after oral administration of drugs. Increase in the foot volume was measured by Plethysmometer (Natume Seisakusho Ltd.) and expressed as percent of the foot volume measured before the injection of carrageenan. Anti-edema effects of the test drugs were expressed in terms of percent inhibition of the foot edema in drug treated group compared with the foot edema in the control group treated with the vehicle.

Anti-inflammatory Activity on Cotton Pellet Granuloma in Rats — The anti-granulomatous effects of test compounds were assayed employing cotton pellet granuloma method.17,18) Male Wistar rats weighing 180—200 g were used. Cotton rolls were made as small segments so that each piece weighed 48—52 mg. After sterilization in an autoclave, 4 cotton pellets were bilaterally implanted subcutaneously on the dosal region. The drugs were administered through stomach tubes once a day for 7 d after the implantation of cotton pellets. After 8th day, the rats were killed and the pellets were carefully re-
moved from surrounding tissues and weighed after being dried over night at 65 °C. The rate of the granuloma formation was calculated as follows:

\[
\frac{\text{dry weight of the granuloma} - \text{initial weight of the cotton pellet}}{\text{initial weight of the cotton pellet}} \times 100
\]

The average of the weights of 4 granuloma was regarded as the weight of granuloma per rat.

**Extraction of Prednisolone and Corticosterone Sample** — Prednisolone and corticosterone sample were extracted according to the method reported in our previous paper. Mice were gentled by daily handling once a day in the morning for 7 d and were decapitated from AM 10:00 to PM 5:00 o’clock. Blood samples were collected and allowed to stand for 1 h at room temperature. After centrifugation at 7400 × g for 10 min, 200 μl of serum were transferred to a 10-ml separating funnel and internal standard solution of dexamethasone (50 ng/5 μl) was added. Then 0.05 ml of 0.25 M sodium hydroxide and 4 ml of methylene chloride were added. The mixture was shaken by hand for 1 min. The organic layer was washed with water, transferred to a 5-ml flask and evaporated in vacuo at 30°C. The residue was dissolved in 100 μl of methanol and 60 μl were injected into the HPLC column.

**Treatment of Shosaikoto and Prednisolone for Measuring Blood Prednisolone Level** — Prednisolone 1.0, 4.0 or 16.0 mg/kg, suspended in 0.2 ml of water and Shosaikoto, 1.1 g/kg, dissolved in 0.2 ml of water were orally given to male ddY mice at the same time. Blood samples were collected by decapitation 30, 60, 120, 180 and 300 min after the administration of Shosaikoto.

**HPLC** — A Shimadzu Model 4A chromatograph with a Shimadzu Model SPD 2A ultraviolet (UV) detector was employed. A stainless steel column (25 cm × 4.6 mm i.d.) packed with reversed phase Hypersil ODS (5 μm, Erma Optical Works Ltd.) was used. The mobile phase was acetonitrile:0.03% sulfuric acid solution (36:64). The column temperature was 50 °C, the flow rate was 1.2 ml/min, detection wavelength was 240 nm and the sensitivity was 0.005 a.u.f.s. Peak area was measured using a Shimadzu C-R2A computing integrator.

**Calibration Graph** — Prednisolone and corticosterone at concentrations varying from 5 to 30 μg per 100 ml and internal standard (dexamethasone) at a fixed concentration of 10 μg per 100 ml were dissolved in 5% albumin solution and a calibration graph was obtained using the procedure described above.

**RESULTS**

*Increase of Prednisolone's Anti-inflammatory Effects by the Combination of Shosaikoto*

Rat carrageenan edema method or cotton pellet method was performed in an experiment of combination of Shosaikoto, 1.1 g/kg, and prednisolone, 1.0, 4.0 or 16.0 mg/kg. Fig. 1 shows the results obtained by carrageenan edema method. Shosaikoto, 1.1 g/kg, showed the tendency of suppressive effect similar to prednisolone, 1.0 mg/kg. Carrageenan edema was suppressed dose-dependently by prednisolone, 1.0, 4.0 and 16.0 mg/kg (Fig. 1 (A)). Combination of Shosaikoto, 1.1 g/kg, promoted edema suppression by the single administration of prednisolone, 1.0 mg/kg, significantly 2 and 3 h after the carrageenan injection (Fig. 1 (B)). Prednisolone, 4.0 or 16.0 mg/kg, combined with Shosaikoto, 1.1 g/kg, resulted in a significant increase in the inhibition of the edema at 7 h or at 5 and 7 h, respectively, as compared with respective groups of single administration of prednisolone (Fig. 1 (C) and (D)).

Fig. 2 shows the results obtained by cotton
FIG. 1. Effects of the Combination of Prednisolone and Shosaikoto on the Swelling of Rat Hind Paw Induced by Carrageenan

Carrageenan (1.0%, 0.05 ml) was injected at 0 h. Prednisolone and Shosaikoto were administered orally 1 h before the injection of carrageenan.

- : control, - - : Shosaikoto (1.1 g/kg), Δ - Δ : prednisolone (1.0 mg/kg), ○ - ○ : prednisolone (4.0 mg/kg), □ - □ : prednisolone (16.0 mg/kg), ▲ - ▲ : prednisolone (1.0 mg/kg) + Shosaikoto (1.1 g/kg), ○ - ○ : prednisolone (4.0 mg/kg) + Shosaikoto (1.1 g/kg), ■ - ■ : prednisolone (16.0 mg/kg) + Shosaikoto (1.1 g/kg). Each point represents the mean of 6–9 male rats. Vertical bars are S.E.M. Statistically significant inhibition to the control group (A) or to the prednisolone treated group (B, C and D) are expressed with marking by a) (p<0.05) or b) (p<0.01).
pellet method. Shosaikoto, 1.1 g/kg, showed ex-uberant suppression of granuloma formation similar to that seen in the prednisolone, 1 mg/kg, treated group, with significant difference from the normal rats.

On the other hand, prednisolone, 1.0, 4.0 and 16.0 mg/kg, suppressed granuloma formation respectively in a dose dependent manner. Combination of Shosaikoto and prednisolone resulted in increased suppression of granuloma formation as compared with prednisolone alone treated groups with statistically significant difference at 5% level for the prednisolone, 4.0 mg/kg, treated group and at 1% level for the prednisolone, 16.0 mg/kg, treated group.

Table 1 summarizes the increase in body weight and the weights of thymus, spleen and adrenal gland measured one week after the administration in the cotton pellet study. Adrenal weight did not decrease in the Shosaikoto, 1.1 g/kg, treated group and prednisolone, 1.0 mg/kg, treated group but decreased significantly in the groups of prednisolone, 4.0 and 16.0 mg/kg. Adrenal weight was not decreased in both of prednisolone, 4.0 and 16.0 mg/kg, treated groups combined with Shosaikoto, 1.1 g/kg. On the other hand, the combination of Shosaiko-

**TABLE 1. Effects of the Combination of Shosaikoto and Prednisolone on Body, Thymus, Spleen and Adrenal Weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increase in body weight (g)</th>
<th>Thymus weight (mg)</th>
<th>Spleen weight (mg)</th>
<th>Adrenal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.7 ± 2.8</td>
<td>577 ± 39</td>
<td>610 ± 44</td>
<td>20.1 ± 1.4</td>
</tr>
<tr>
<td>Shosaikoto (1.1 g/kg)</td>
<td>40.8 ± 4.2</td>
<td>570 ± 40</td>
<td>637 ± 60</td>
<td>20.0 ± 0.6</td>
</tr>
<tr>
<td>Prednisolone (1.0 mg/kg)</td>
<td>35.0 ± 4.3</td>
<td>517 ± 34</td>
<td>613 ± 34</td>
<td>19.7 ± 1.0</td>
</tr>
<tr>
<td>Prednisolone (1.0 mg/kg) + Shosaikoto (1.1 g/kg)</td>
<td>38.3 ± 2.1</td>
<td>463 ± 41</td>
<td>584 ± 10</td>
<td>17.8 ± 0.8</td>
</tr>
<tr>
<td>Prednisolone (4.0 mg/kg)</td>
<td>40.5 ± 3.2</td>
<td>258 ± 8</td>
<td>464 ± 49</td>
<td>16.3 ± 0.9</td>
</tr>
<tr>
<td>Prednisolone (4.0 mg/kg) + Shosaikoto (1.1 g/kg)</td>
<td>32.5 ± 2.8 $^a$</td>
<td>291 ± 25</td>
<td>467 ± 36</td>
<td>19.3 ± 0.9</td>
</tr>
<tr>
<td>Prednisolone (16.0 mg/kg)</td>
<td>25.8 ± 5.7 $^a$</td>
<td>206 ± 25</td>
<td>466 ± 20</td>
<td>16.0 ± 0.7 $^a$ $^p &lt; 0.05$</td>
</tr>
<tr>
<td>Prednisolone (16.0 mg/kg) + Shosaikoto (1.1 g/kg)</td>
<td>32.5 ± 1.7 $^a$</td>
<td>197 ± 26</td>
<td>406 ± 18</td>
<td>19.1 ± 1.2</td>
</tr>
</tbody>
</table>

*Shosaikoto and prednisolone were administered orally for 7 d. Each value is the mean of 6 male rats with S.E.M. Significant difference to the control group is expressed with marking by a) ($p < 0.05$) and b) ($p < 0.01$).*
to, 1.1 g/kg, showed no significant effects on the decrease of body, thymus and spleen weight induced by prednisolone, 1.0, 4.0 or 16.0 mg/kg.

**Effects of Shosaikoto on the Blood Prednisolone Level**

To investigate the detail mechanism of the action of Shosaikoto which increases the anti-inflammatory action of prednisolone, blood prednisolone level was determined by HPLC when Shosaikoto was combined with prednisolone. Fig. 3 shows the blood prednisolone level when prednisolone, 1.0, 4.0 and 16.0 mg/kg, were combined with Shosaikoto, 1.1 g/kg, by oral administration. Blood prednisolone level was approximately doubled by the combination of Shosaikoto, 1.1 g/kg, with prednisolone, 16.0 mg/kg, 20 min after the administration. By the combination of Shosaikoto, 1.1 g/kg, and prednisolone, 1.0 or 4.0 mg/kg, the blood prednisolone level was not increased. The half life period of blood prednisolone was about 2 h after the single administration of prednisolone, 16.0 mg/kg, and that was 1 h after the combined use of Shosaikoto, 1.1 g/kg, and prednisolone, 16.0 mg/kg.

**Effects of Shosaikoto on the Blood Corticosterone Level**

To investigate whether the anti-inflammatory effect of Shosaikoto is due to the stimulation of pituitary-adrenocortical axis function or not, blood corticosterone level was determined by HPLC. Fig. 4 summarizes the results. Corticosterone level increased 1 h after the p.o. treatment of Shosaikoto, 1.1 g/kg. At 3 h after its administration, blood corticosterone decreased to minimum level and in 60% of mice used, corticoste-

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**FIG. 3. Effects of the Combination of Prednisolone and Shosaikoto on the Blood Prednisolone Level**

Prednisolone and Shosaikoto were administered orally at 0 h. △——△ : prednisolone (1.0 mg/kg), △——△ : prednisolone (1.0 mg/kg) + Shosaikoto (1.1 g/kg), □——□ : prednisolone (4.0 mg/kg), □——□ : prednisolone (4.0 mg/kg) + Shosaikoto (1.1 g/kg), ○——○ : prednisolone (16.0 mg/kg), ○——○ : prednisolone (16.0 mg/kg) + Shosaikoto (1.1 g/kg). Each point represents the mean of 7—8 male mice. Vertical bars are S.E.M. Statistically significant difference to each prednisolone treated group is expressed with marking by a) (p < 0.05) or b) (p < 0.01).

**FIG. 4. Effects of Shosaikoto on the Blood Corticosterone Level.** Shosaikoto was administered orally at 0 h. ○——○ : control, ●——● : Shosaikoto (1.1 g/kg). Each point represents the mean of 10—19 male mice. Vertical bars are S.E.M. Statistically significant difference to the control group is expressed with marking by a) (p < 0.01).
rnone was not detected. After 5 h, the corticosterone level reached to the control level.

DISCUSSION

It seems considerably difficult to elucidate the mechanism of action of Kampohozai (Chinese traditional medicine) such as Shosaikoto which is a mixture of natural substances. This constitutes the greatest problem for making use of the Kampohozai in modern medicine, but we cannot neglect the piles of clinical experiences in Kampohozai for more than 2000 years. And that, Kampohozai was reported to be effective for chronic inflammatory disease including hepatitis etc. Recently. For the purpose of bringing up the Kampohozai to the level of western medicine recognition, fundamental experiments have to be carried out for factual accumulation.

As the first step toward this purpose, we employed carrageenan edema and cotton pellet methods which are general screening methods for anti-inflammatory effects. The results obtained in carrageenan edema show that Shosaikoto, 1.1 g/kg, increased the anti-inflammatory action of prednisolone additively. In cotton pellet method, the action of prednisolone was markedly intensified by the combination of Shosaikoto, 0.11 g/kg. In both experimental model, effects of prednisolone were not increased by the combination of Shosaikoto, 0.11 g/kg which corresponded to the human dose/d.

In the case of the combined p.o. administration, Shosaikoto is expected to possess two mechanism of increasing the anti-inflammatory action of prednisolone. One possibility is to accelerate the absorption of prednisolone into blood by Shosaikoto which contains saponins and starch etc. and behaves as soluble reagents. Blood prednisolone level was evaluated 10–50 min after the combination of prednisolone and Shosaikoto by the oral administration. The blood prednisolone level after the combined administration of prednisolone with Shosaikoto was about two times that of the single use of Shosaikoto. This apparent phenomenon was observed in the high dose of prednisolone, 16.0 mg/kg. The pattern of the blood concentration curve of prednisolone, 16.0 mg/kg, was different from those of 1.0 and 4.0 mg/kg. The peak was broad and its half-life was about two times as those of 1.0 and 4.0 mg/kg. These results suggest that the dose of 16.0 mg/kg in mice exceeds the permeability of intestine or the ability of metabolism. Judging from the unchanged blood prednisolone level at 1.0 and 4.0 mg/kg in the combination with Shosaikoto, the synergism observed in carrageenan-induced edema and cotton pellet granuloma test can not be explained. However, in the combination of prednisolone, 16.0 mg/kg, and Shosaikoto, 1.1 g/kg, the increased anti-inflammatory action may be explained partially by the increased blood prednisolone level.

Another possibility is that Shosaikoto possesses anti-inflammatory effect and increases the action of prednisolone additively. In carrageenan edema and cotton pellet method, Shosaikoto showed anti-inflammatory effect which is about the same to that of prednisolone, 1.0 mg/kg. Samnels et al., Rousseau et al., Baxter et al. and Tsuruju et al. reported that glucocorticoid receptor and the induction of a gene expression are involved in the anti-inflammatory action of glucocorticoids. Using the method reported by Tsuruju, we obtained the results that one of the mechanisms of anti-inflammatory action of Shosaikoto was steroidal.

On the other hand, stimulating effect of Shosaikoto on the pituitary-adrenocortical axis is expected by the reason that the single p.o. administration of Shosaikoto increased the blood corticosterone level and combined use of Shosaikoto with prednisolone inhibited the decrease of adrenal weight induced by prednisolone with a significant manner in cotton pellet experiments. Therefore, the elevated blood level of endogenous glucocorticoid also can be expected to show an anti-inflammatory action. As to the increase of blood corticosterone level by the p.o. administration of Shosaikoto, it is possible that Shosaikoto inhibits the reduction of glucocorti-
coid in liver in addition to its stimulating effect on the pituitary-adrenocortical axis. This action may be explained by glycyrrhetinic acid,28,29 aglycones of glycyrrhizin involved in Glycyrrhizae Radix and other triterpenes and sterols which are involved in Shosaikoto.

In our experiments, whether the increased anti-inflammatory action of prednisolone by the combination of Shosaikoto is caused by the addition or the potentiation of both effects of prednisolone and Shosaikoto can not be clarified. However, our results show that Shosaikoto involves the ability to increase the anti-inflammatory action of prednisolone with the inhibition of the decrease of adrenal weight induced by prednisolone. But, the decrease of thymus and spleen weights induced by the single administration of prednisolone was not changed by the combination with Shosaikoto with significant manner. Detail investigations of Shosaikoto on the immune response will be needed in future. To clarify the mechanism of these synergism recognized by the combination of prednisolone and Shosaikoto in the anti-inflammatory action, the active principles fractionated from the extracts of Shosaikoto should be studied pharmacologically.

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